BANANA GUARD

Protocols

Assembly of new promoters from oligo's:

Oligo's Klenow Polymerase protocol:

- Make 10 uM stock from 100 uM stock
- Mix the following in a 0.6 mL sterile tube
  10 µL 10X NEBuffer 2.1 (containing BSA)
  4 µL oligo 1 10 uM (typically 1 µg or more)
  4 µL oligo 2 10 uM (typically 1 µg or more)
  75 µL deionized sterile H_2O
- Anneal the two oligos together by either placing the mixture in a thermal cycler (MJ Research, PTC-200) at 94°C for 5 mins, a cool down for 0.1°C/sec to 5°C below the melting temperature of the primers, hold that temperature for 5 mins, then cool down at 0.1°C/sec to 37°C. Alternatively, the tube can be placed in a beaker of boiling water and let cool to room temperature.
- Add 1 µL Klenow 3'⟶ 5' exo$^-$ polymerase to mixture.
  Vortex polymerase before pipetting to ensure it is well-mixed.
- Add 2.5 µL 10mM dNTPS (equal to 0.25 mM final concentration of each dNTP).
  Recommend using a thermal cycler for the following incubation steps.
- Incubate 1 hr at 37°C.
- Heat inactivate polymerase by incubating at 75°C for 20 minutes. This inactivation temperature might be higher than the melting temperature of your annealed and extended primers. It may be prudent to ramp the temperature down from 75°C.
  See Restriction Digest for more information on the following steps.
- Add 1 µL EcoRI and 1 ul SpeI
- Incubate for a minimum of 2 hrs.
- Heat inactivate restriction enzyme by incubating at 80°C for 20 mins.
- Purify DNA as necessary and measure concentration with nanodrop